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CONTRAST ENHANCED ULTRASONOGRAPHY OF THE DOG'S SPLEEN

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ABSTRACT

The present research aims to visualize and follow the arterial and venous blood vessels pattern and to detect the circulatory phases of the canine spleen. Six dogs included in the study were examined with conventional and contrast ultrasonography using SonoVue® (Bracco International B.V., Nederland) contrast agent. The main arterial and venous vessels were observed. The timing of the different circulatory phases was detected and showed that the contrast–enhanced ultrasonography of the canine spleen should be carried out immediately after the introduction of the contrast agent and should be performed not later than the 3rd minute.

Key words: spleen, blood supply, contrast ultrasonography, SonoVue®.

Introduction

The spleen is the biggest and the best blood–supplied lymph organ in mammals (Vodenicharov, 2021) and is the peripheral organ of the hemopoietic and immune system (Sapundziev & Chervenkov, 2020). The spleen is often involved in different pathological conditions, and it could be considered as a main reason for some neoplastic diseases (Valli *et al.*, 2016).

Imaging diagnostics and ultrasound methods for examination of the internal organs are a major part of veterinary practice, especially in the last quarter of the century (Mattoon & Nyland, 2015). At the same time, the blood vessels of the spleen are poorly investigated with contrast computed tomography or ultrasonography, which gave the authors a reason for further studies. In veterinary, so as in human medicine, the spleen is widely investigated by ultrasonography (Soyupak *et al.*, 2002; O'Brien *et al.*, 2004; Catalano *et al.*, 2005; Stefanello *et al.*, 2009; Herbay *et al.*, 2009; Nakamura *et al.*, 2009; Popescu *et al.*, 2009; Taeymans & Penninck, 2011; Dimitrov *et al.*, 2012; Maronezi *et al.*, 2015; Omar & Freeman, 2016; Rossi *et al.*, 2016; Young Choi *et al.*, 2016; Sutil *et al.*, 2017; Shabani *et al.*, 2018; Mosallanejad *et al.*, 2018; Lerchbaumer *et al.*, 2019). The new technologies in the ultrasound technique combined with the new generations of contrast agents allow a significant increase in the informativeness of the method and expand the field of use of ultrasonography in medical practice. This makes contrast–enhanced ultrasound (CEUS) an alternative method to other imaging methods (Piscaglia *et al.*, 2012; Lim *et al.*, 2004; Sammon *et al.*, 2012). With it, the parenchymal structure of organs such as the liver, spleen, pancreas, and an evaluation of their condition is given by intravenous injection of a contrast agent. It can also be used to detect lesions with a density similar to the normal ultrasound density of the organ (Piscaglia & Bolondi, 2006). Unlike computed tomography, the CEUS is not associated with radiation exposure to the patient. It lacks the risk of allergic reactions, it is not associated with pain, and in most cases, it could be performed on non–anesthetized patients (Piscaglia *et al.*, 2012; Lim *et al.*, 2004; Sammon *et al.*, 2012). Despite the greater number of scientific reports on the application of the CEUS method in human medicine, in veterinary practice, its application is still limited so as the number of scientific reports on its use (Piscaglia *et al.*, 2012; Lim *et al.*, 2004; Sammon *et al.*, 2012).

The ultrasound microbubbles are unique among radiographic contrast agents due to their ability to remain entirely within the intravascular space after injection. The superficial location of the spleen in the dog makes it even more convenient for contrast–enhanced ultrasound examination (Piscaglia *et al.*, 2012; Lim *et al.*, 2004; Sammon *et al.*, 2012). Lim *et al.* 2004 observed the pharmacokinetics of a microbubble contrast agent in humans. They stated that hyperechogenicity persists long after the contrast agent has been eliminated from other organs. This feature is particularly pronounced in the spleen, and hyperechogenicity often persists for 5–7 minutes after injection (Haers *et al.*, 2009), making it ideal for CEUS studies.

The aim of the present research was to detect and trace the arterial and venous blood vessels of the spleen as well as its parenchymal view with the use of contrast and conventional ultrasonography. A timing observation of the circulatory phases was also performed.

Materials and methods

The data for the study was collected over the period of 2 years during contrast–enhanced ultrasonography, performed for clinical reasons not related to the spleen. Three female and three male mixed breed dogs were examined, with an age from 1 to 3 years and a weight of 6–15 kg. The ultrasound was performed with Z50 Vet Mindray (Mindray Global, Nanshan, Shenzhen, China) – a veterinary sonographer. A micro convex transducer with a frequency of 3.5–8 MHz and 8–9 MHz with harmonics was used. The patients were fixed in a right lateral recumbent position, the hair cover was removed in the left lateral abdominal, umbilical, and xiphoid region and Eco Gel–200 was applied to the skin.

The method is based on the venous application of an ultrasound contrast, a dispersion solution of microbubbles smaller than the size of erythrocytes (<6.2 до 8.2 μm). The second–generation contrast agent SonoVue® (Bracco International B.V., Nederland) was introduced through a 20G venous catheter placed in the cephalic vein, with a dosage of 0.05 ml/kg body weight, followed by 2 ml of 0.9% NaCl. When the microbubbles reach the organ, they reflect the ultrasound wave to a greater value and thus amplify the image and improve it. After the introduction of the contrast agent, the organ was monitored, and the different phases of the contrast passage were detected. A split screen was used in which a conventional grayscale image was displayed alongside the CEUS image allowing constant comparison of the echogenicity. The measurement of echogenicity enhancement of the spleen was carried out in grayscale in a B–mode imaging view, which provides a dynamic image in real–time (Nakamura *et al.*, 2009). All terms are unified with Nomina Histologica Veterinaria (Seeger *et al.*, 2017) and Nomina Anatomica Veterinaria (Simoens *et al.*, 2017).

Results

On percutaneous transabdominal ultrasonography, the spleen of the dog is usually compared with the cortex of the left kidney, as the spleen has a hyperechoic appearance and a fine–grained, homogeneous echotexture (Fig. 1). As a histological structure of the spleen, the hyperechoic capsule (*tunica fibromuscularis*) could be visualized with conventional ultrasound, due to its content of two–layered fibrous unformed tissue, which explains the hyperechoic appearance of the *trabeculae splenicae* penetrating its interior. With this type of ultrasound, the trabecular veins (*venae trabeculares*) were observed (Fig. 1). Before the contrast administration, the spleens of all dogs had a hyperechoic structure compared with the cortex of the left kidney.

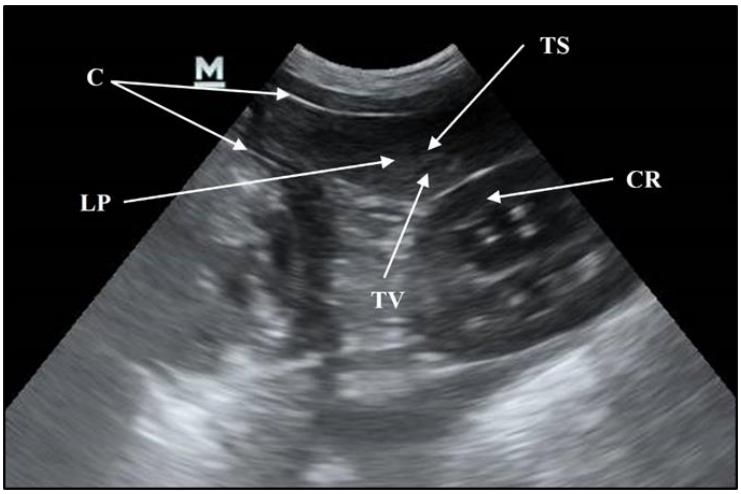

Figure 1: Conventional ultrasonography of the abdominal organs, the transverse–sagittal position of the transducer: C – *tunica fibromucularis* (*capsula*); LP – hyperechoic spleen parenchyma; TS – *trabecula splenica*; TV – *vena trabecularis*; CR – *cortex renalis* of the left kidney.

After the contrast administration, the echoic enhancement of the organ started at the 16th second on average and reached its maximum around the 30th second. Arterial blood brings microbubbles and distributes them in the spleen during the arterial phase (wash–in phase), but they cannot continue their passage into the venous system immediately. This gives the parenchyma of the organ a hyperechoic inhomogeneous view, with numerous anechoic and hypoechoic small oval areas. That could be explained by the closed type of blood circulation of the spleen in the dog. During the arterial phase, the contrast enters the *sinus venularis* (*lienis*, *venosus*), where the physiological sphincter of its efferent part does not let it pass along with the masses of erythrocytes. At the same time, the sinuses are saturated, filled, and overflowed, and at this point *v. pulpae rubrae* remain empty of contrast medium, which gives the look of the described anechoic oval areas. During this arterial phase, the so–called "zebra view" (Fig. 2) was observed until the 30th second on average.

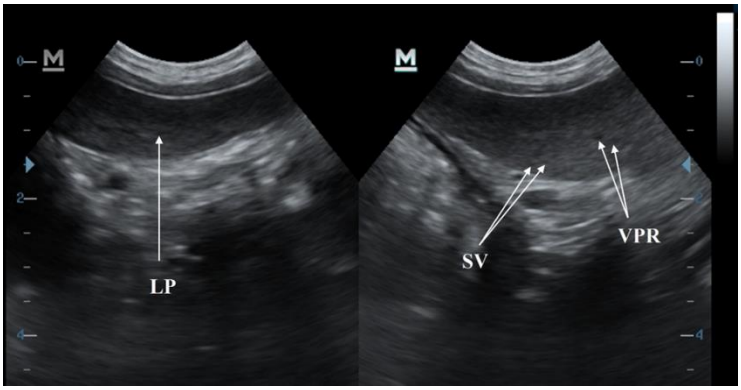

Figure 2: A comparison of CEUS view at the beginning of the arterial phase at the 16th second (left) and at the 29th second (right); transverse position of the transducer LP– hyper echogenicity of the spleen parenchyma; SV – *sinus venularis* (*venosum*, *lienalis*) full of contrast agent; VPR – *venae pulpae rubrae*.

Later the contrast agent continued to the venous vessels the hypoechoic areas decreased in size and number until the organ acquired a homogeneous hyperechoic appearance, also called the parenchymal phase. This can be explained by the passage of the contrast through the physiological sphincter of the venous sinus to the veins of the red pulp on the one hand and the residual amounts of contrast in the sinuses on the other. This phase was established at 35–120 seconds after the contrast introduction. After the parenchymal phase, a decrease in echogenicity started, which shows a wash–out phase of the contrast.

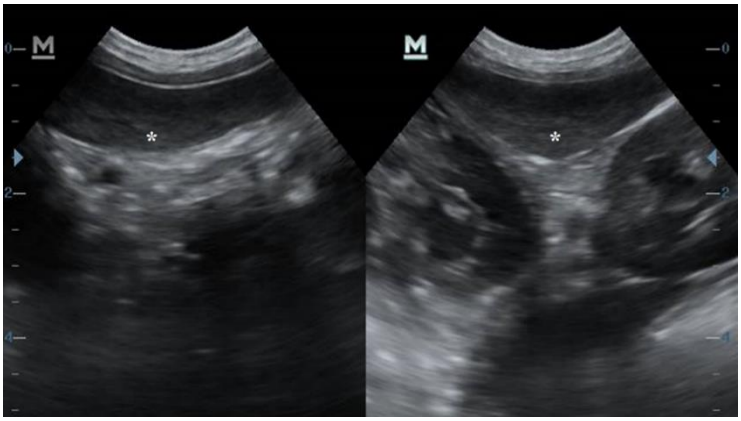

Figure 3: CEUS of the spleen with a transverse position of the transducer. Homogeneous hyperechogenicity corresponding to the venous phase was observed to about 40–60 seconds after the administration of the contrast agent.

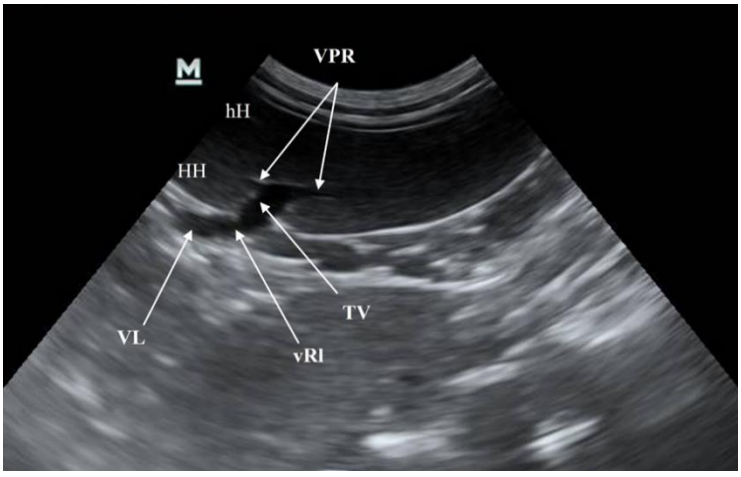

Figure 4: CEUS of the spleen 1 minute after the administration of the contrast with a transverse position of the transducer. VPR – *venae pulpae rubrae*; TV – *vena trabecularis*; vRl – *ramus lienalis* (*venosus*); VL – *v. lienalis*; hH – hypoechoic zone of vessels empty of contrast; HH – hyperechoic areas containing residual contrast.

After the 120th second the contrast substance started to drain strongly through the venous system, at first through the veins of the red pulp (*v. pulpae rubrae*), which form the trabecular veins, and then in the venous *rami lienalis* to inflow into the splenic vein (Fig. 4). This coincides with vigorous pumping of contrast to the splenic vein, which drains into the portal vein. During this phase, hyperechoic heterogeneity is again observed, which is due to residual amounts of contrast in the

venous sinuses and veins of the red pulp and is visualized as hyperechoic shadows. Hypoechoic oval shadows are due to the leaving contrast from the terminal portions of the microcirculatory vessel network. The retention of this phase in our studies was up to 2 minutes.

Between the 2nd and the 3rd minute powerful evacuation of the contrast occurred. Its hyperechoic homogeneous echotexture restored its sonographic appearance and is nearly hyper–isoechoic to the pre–contrast images. Figure 5 shows the comparison of the echotexture of the spleen before (A), during (B), and after the evacuation (C). After the indicated time interval, even if there were remnants of SonoVue, this did not lead to a change in the homogeneous shadow of the ultrasound image of the spleen.

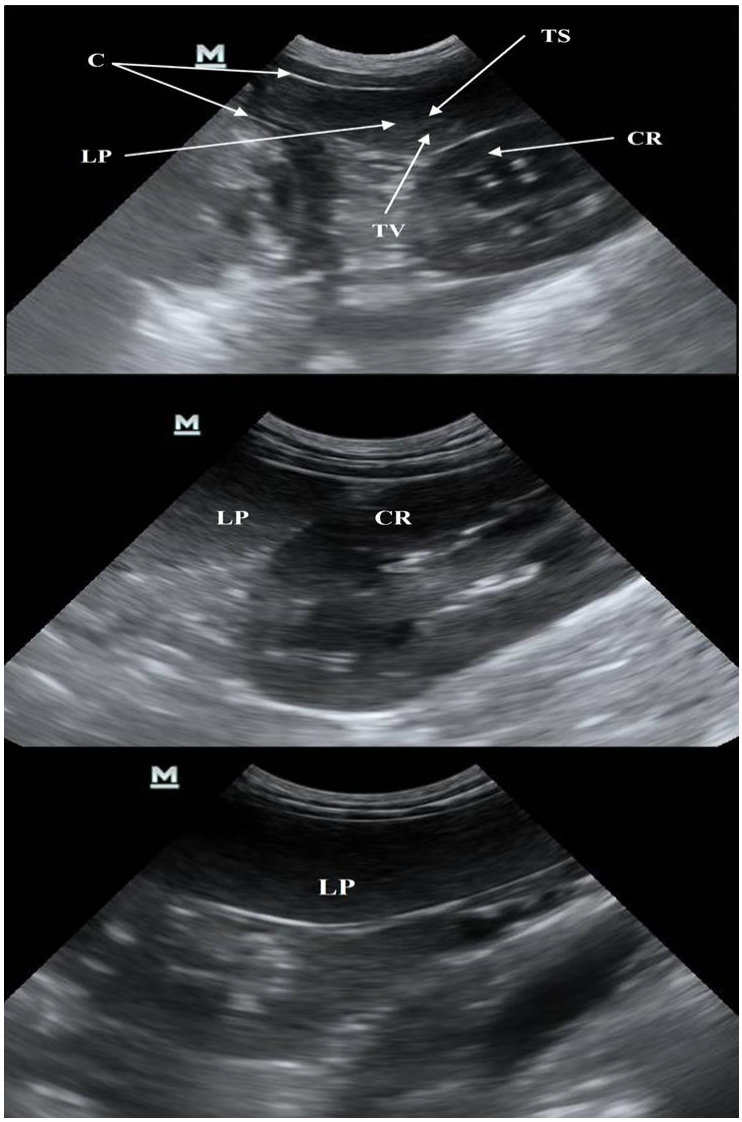

Figure 5: Ultrasonography of the abdominal organs before the evacuation of the contrast (A), during the evacuation on average 2–3 minutes (B) and of fully evacuated contrast (C); transverse–sagittal position of the transducer. C – *tunica fibromucularis* (*capsula*); LP – hyperechogenicity of the spleen parenchyma; TS – *trabecula splenica*; TV – *vena trabecularis*; CR – *cortex renalis* of the left kidney.

Discussion

The normal echogenicity of the spleen on conventional ultrasonography in a comparative aspect with the left kidney confirms the described by Nyland, T. G., & Mattoon, J. S. (2015). The timing of the first changes – the beginning of the arterial phase, confirms the established data in the dog (Nakamura *et al.,* 2009, Ohlerth *et al.,* 2007, Haers *et al.*, 2009). The arterial phase with a hyperechoic non–homogeneous diffuse shadow lasts on average between the 16th and the 29th second, which matches the 19.4 seconds reported by Ohlerth *et al.* (2007) and 18.5 seconds by Maronezi *et al.* (2015), but is earlier than the 45th second according to Taeymans & Pennink (2011). During this phase, the blood brings the microbubbles to the organ, but they cannot pass directly to the venous system, which was also described in the liver (Nyman *et al.*, 2005) and kidneys (Choi *et al.*, 2016). The appearance of a "zebra" view at the end of the arterial phase is a confirmation of the described by Haers *et al.* (2009), Canejo–Teixeira *et al.* (2022), and Maronezi *et al.* (2015) and earlier that the effect in the human that occurs at about 50th second or a minute after according to Omar & Freeman (2016).

The swirling and delayed passage of the microbubbles is evidence of the physiological sphincter existence at the efferent part of the venous sinuses, which reconfirms the report by Harmanson *et al.* (2020). The parenchymal, venous phase, in which homogeneous hyperechogenicity (Fig. 3) is observed between the 40th and 120th second is in confirmation of those measured by Ohlerth *et al.* (2007) in large breed dogs, and in humans by Herbay *et al.* (2008) and Popescu *et al.* (2009), while Haers *et al.* (2009) observed it for 5 to 7 minutes in dogs. The cycle of passage from venous sinuses to red pulp veins was also established in accordance with Harmanson *et al.* (2020). No change was found in the shape and size of the spleen after the administration of Sono Vue contrast (Canejo–Teixeira *et al.*, 2022).

The evacuation of contrast material from the splenic parenchyma started between the 2nd and the 3rd minute, while Haers *et al.* (2009) established it after the 5th – 7th minute. It is possible to retain contrast for a longer time due to the cyclicity in the passage through the venous sinuses to the veins of the red pulp from a few minutes to 10 hours (Harmanson *et al.*, 2020). This occurs at most 7–30 minutes after intravenous application according to Nakamura (2009) and Haers *et al.* 2009. Prolonged duration was not detected in our studies. The effect of restoration of the ultrasonographic image of the spleen in our studies occurred much earlier, on average, around the 3rd minute, and no contrast swirling and delay were observed.

Conclusion

The conducted contrast–enhanced ultrasound established the main nutritive vessels of the dogs' spleen, as well as the phases of the arterial and venous circulation and their duration, which is related to the canine closed type of blood circulation. By the observed results the authors can conclude that the contrast–enhanced ultrasonography in dogs should be carried out continuously, right after the contrast administration and not later than the 3rd minute.

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